

TECHNICAL AMENDMENTS TO THE SPECIFICATION:

Please insert the following text into the specification between lines 3 and 4:

--CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of pending U.S. Patent Application Serial No. 09/937,236, filed on December 26, 2001, which is a U. S. national stage filing of International Patent Application Ser. No. PCT/FR00/00713, filed March 22, 2000, which claims priority to French Patent Appl. Ser. No. 99/03,701, filed March 22, 1999, the contents of which are incorporated herein in their entireties.—

Please amend the paragraph beginning at page 30, line 10 and ending at page 33, line 4 with the following rewritten paragraph:

Protoplasts of the G11.174 strain were cotransformed with pNi160 plasmid (introducing *Impala 160*) and the pCB1179 plasmid (Sweigard et al., 1997) conferring hygromycin resistance. The transformation method is described by Sweigard et al., 1992 and was carried out in the presence of 4 units of BamHI enzyme (REMI: restriction enzyme mediated integration; Sweigard et al., 1998) and 1 μg of each plasmid. The protoplasts are selected on a TNKYE medium in which the glucose has been replaced with sucrose ($400 \mu\text{g.l}^{-1}$), and supplemented with hygromycin in a proportion of $240 \mu\text{g.ml}^{-1}$. In order to select the cotransformants, the colonies resistant to this antibiotic were analyzed, after extracting their genomic DNA, by amplification using the SPE5 (5'AGAACACAACCCTGCCACGG3')(SEQ ID NO:1) and SPE3 (5'TCCGGGCGGTATGCACAGAG3')(SEQ ID NO:2) primers which are specific

for the *Impala* transposon and which generate a 573 bp amplification product. The cotransformant DNA was digested with EcoRI and analyzed by Southern blot (figure 3) using, as a probe, a 2.7 kb EcoRI fragment of the *niaD* gene (2.7 kb probe) present in pAN301 (Malardier et al., 1989). This study made it possible to select 35 cotransformants having at least one 4 kb band representing virtually the entire *nia* gene from *Aspergillus nidulans* introduced via pNi160. These cotransformants were cultured on riceflour-based solid medium for 10 to 14 days, and the spores were harvested in water. After counting, they were seeded onto MNO₃ agar medium in a proportion of 10⁵-10⁶ spores per dish. Experiments reconstituting this step for selecting the *nia*⁺ revertants were carried out. We thus observed that the MNO₃ medium makes it possible to detect *nia*⁺ colonies when 10 wild-type (G11.25) spores are mixed with 10⁶ spores of the *nia*⁻ G11.174 mutant and incubated for 14 days at 26°C. After culturing for 1 month at 26°C, only one cotransformant (cotransformant C14) made it possible to obtain two colonies (C14-1 and C14-2) with an aerial phenotype. These revertant colonies were recovered and analyzed by PCR using the C1 (5'CGCTGCGAATTCTTCAGT3')(SEQ ID NO:3) and *niaX* (5'CTAGACTTAGAACCTCGG3')(SEQ ID NO:4) primers framing the *Impala* 160 insertion site in the promoter of the *niaD* gene. The amplification of a 200 bp product reveals the presence of nuclei in which the excision of the transposon has taken place. In order to obtain homogeneous colonies, conidia of the C14-1 and C14-2 revertants were isolated under a binocular magnifying glass and cultured separately. The analysis thereof by Southern blot, using a probe corresponding to

the ORF of *Impala* makes it possible to demonstrate the reinsertion of the element in the two revertants (figure 4). The footprint left by the excision of the transposon was sequenced after cloning the 200 bp PCR product into the pGEM-T easy vector (Promega). The footprint of the C14-1 revertant is CTGTA and that of C14-2 is CAGTA. These footprints are identical to those most commonly left by *Impala* when it is excised in *Fusarium oxysporum* (Langin et al., 1995). In culture on MNO₃ agar medium, these revertants have an intermediate phenotype which is between that of the G11.174 and G11.25 strains, suggesting that the *niaD* gene present in the pNi160 construct does not allow optimal complementation of the mutation of the G11.174 strain. In order to test this hypothesis, protoplasts from this strain were transformed with the pAN301 (3 µg) or pAN301ΔNdeI (3 µg) vectors containing the *niaD* gene under the control of 1.3 kb of promoter, and in the presence of pCB1179 (3 µg). After plating the protoplasts out and incubating at 30°C for 10 days, on MNO₃ medium supplemented with hygromycin (240 µg.ml⁻¹) and in which the glucose has been replaced with sucrose (400 µg.l⁻¹), colonies with an *nia*⁺ phenotype appear. On the other hand, no complementation was observed when the p11ΔNdeI vector containing the *niaD* gene under the control of a 0.3 kb promoter fragment (as in the case of pNi160) was used.

Please amend the paragraph beginning at page 35, line 19 and ending at page 36, line 19 with the following rewritten paragraph:

The observation of 350 revertants generated from CTRF6 made it possible to detect a revertant (Rev2) exhibiting, on rice medium and NaNO₃

medium, less significant growth than CTRF6, and also a dark brown coloration which is different from the gray color of the cotransformant. In order to characterize the sequences flanking the *Impala* insertion, 3 µg of Rev2 genomic DNA were digested with HindIII and analyzed by Inverse-PCR. After digestion, the DNA is subjected to a phenol/chloroform extraction step and then precipitated with 7.5M ammonium acetate. The pellet thus obtained is taken up in 40 µl MilliQ water. A ligation is carried out on 8 µl of digested DNA and then the DNA is subjected, as previously, to phenol/chloroform extraction and precipitated with 7.5M ammonium acetate. The DNA is taken up in 10 µl, all of which is used for a PCR step using the ImpE5' (5'GGCATTGAAAACGCGGTCCC3')(SEQ ID NO:5) and ImpE3' (5'CAGCAGCAAAACAGCTGCCC3')(SEQ ID NO:6) primers which are chosen on the sequence of the *Impala* transposon and which are positioned divergently. The sequencing of the IPCR product made it possible to show that the transposon is inserted into an open reading frame at a TA dinucleotide which is duplicated. Examination of the databanks using the tblastx program (Altschul et al., 1990) revealed very strong homology between this mutated sequence and a protein family involved in DNA repair, and more particularly with the MLH1 protein of *Saccharomyces cerevisiae* (Prolla et al., 1994).

Please amend the paragraph beginning at page 44, line 5 and ending at page 45, line 15 with the following rewritten paragraph:

Protoplasts from *Magnaporthe grisea* G11.174 were transformed with the pHNiL plasmid or cotransformed with the pHNiL and pEO6 plasmids. The transformation method is described by Sweigard et al. (1992) and was carried out with 1 μ g of each plasmid. The protoplasts are selected on a TNKYE medium in which the glucose has been replaced with sucrose (400 μ g.l⁻¹), supplemented with hygromycin in a proportion of 240 μ g.ml⁻¹. The pHNiL transformants are directly selected by virtue of the presence of the resistance marker in the defective element. The cotransformants are isolated from the hygromycin-resistant colonies, after extraction of their genomic DNA, by amplification using the SPE5 primers described in IV.3. This study carried out on 12 hygromycin-resistant colonies allowed 4 colonies also carrying pEO6 to be isolated. After sporulation on riceflour-based solid medium, the spores (10^5 - 10^6) of these cotransformants, and also of 6 transformants carrying pHNiL, were plated out on MNO₃ medium in order to select nia⁺ revertants as described in IV.3. None of the 6 transformants carrying pHNiL gave such revertants. This shows that the defective copy of *Impala* cannot be mobilized by a transposon endogenous to *Magnaporthe grisea*. Among the 4 pHNiL/pEO6 cotransformants, two of them give aerial colonies (cotransformants D1 and D9). The Southern analysis of 6 revertants derived from the D1 cotransformant, after digestion of their genomic DNA with EcoRI and hybridization with an 868 bp probe from the *hph* gene, obtained using the hyg1 (5'AGCCTGAACTCACCGCGACG3')(SEQ ID NO:7) and hyg4 (5'CGACCCTGCGCCCAAGCTGC3')(SEQ ID NO:8) primers, makes it possible to characterize the reinsertion of the defective element for 4 of them (figure 14).

Among the latter, two revertants contain two insertions of the element. This analysis makes it possible to show that the defective element can be mobilized, in *Magnaporthe grisea*, by the *Impala* transposase provided in trans.

Please insert the paper copy of the Sequence Listing enclosed herewith into the instant specification after the Abstract of the Disclosure on p. 56. The Sequence Listing thus becomes pp. 57-59 of the specification.